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THE *MRJP3* MICROSATELLITE MARKER: DETERMINATION OF HONEYBEE SUBSPECIES OR/AND ROYAL JELLY PRODUCTIVITY OF BEE COLONY

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Summary. The *mrjp3* gene is a member of the *mrjp*-family that encodes Major Royal Jelly Proteins in bees. In the structure of the *mrjp3* gene coding part, a repetitive region, designated as a microsatellite *mrjp3* locus, is described. The variability of the *mrjp3* microsatellite locus in 575 honeybees from Siberia (Russia) was studied. In honeybees of different origin (evolutionary branches M and C) inhabited Siberia, the differences in the frequency of allele registration were revealed. The significance of the *mrjp3* locus for determining the honeybee subspecies and/or royal jelly productivity of the bee colonies is discussed.

Key words: *Apis mellifera,* honeybee, microsatellite *mrjp3* locus, royal jelly, subspecies, productivity.

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Резюме. Ген *mrjp3* – член *mrjp*-семейства, кодирующего основные белки маточного молочка у пчёл. В структуре кодирующей части гена *mrjp3* описан повторяющийся район, обозначенный как микросателлитный локус *mrjp3*. Вариабельность микросателлитного *mrjp3* локуса была изучена у обитающих в Сибири 575 медоносных пчёл. У медоносных пчёл различного происхождения (эволюционные линии M и C) были выявлены различия в частоте регистрации аллелей. Обсуждается значение *mrjp3* локуса для определения подвидов медоносных пчёл и/или продуктивности маточного молочка пчелиных семей.

INTRODUCTION

Royal jelly (RJ) is secreted by the hypopharyngeal and mandibular glands of nurse bees and plays an important role in honeybee development and determination of the reproductive status of a caste (Buttstedt *et al*., 2014). RJ is a complex mixture of water, lipids, sugars, proteins, low molecular mass compounds, salts, and other minor components (Sabatini *et al*., 2009). About 90% of all proteins are major royal jelly proteins (MRJPs) (Schmitzová *et al*.,

1998). Due to its biological properties, RJ is a commercial product widely used in the pharmaceutical, food, cosmetic, and other industries.

RJ production is influenced by many factors such as posture and acceptance of larvae, size and bursa number of the hypopharyngeal glands, the time and the period of RJ secretion, genetic features of bees, and the environmental factors including nutrition, climate conditions, and others (Cao, 2016; Ostrovski-Tomporoski *et al*., 2016). For example, the content of 10-hydroxy-*trans*-2-decenoic acid, which is the most important RJ quality criteria, varies significantly with season, harvesting time (Zheng *et al*., 2011), breed, and geographical origin of bees (Wei *et al*., 2013).

Traditionally, studies of RJ are mainly concerned with biochemical aspects such as the parameters investigated concern the organoleptic characteristics, physicochemical properties, and compositional factors (Sabatini *et al*., 2009). Morphological and cytological features as well as differential expression of proteins in hypopharyngeal glands are also being studied (Cao *et al*., 2016). However, genetic assessments of bee colonies, for example, an analysis of the level of RJ productivity in honeybees of different subspecies (different evolutionary branches) or the genetic characteristics of highly RJ productive bee colonies, are rare (Chen *et al*., 2005; Ji *et al*., 2009; Baitala *et al*., 2010; Yin *et al*., 2011; Parpinelli *et al*., 2014; Ruvolo-Takasusuki *et al*., 2016). At the same time the selection of bee colonies (breeds) with a high RJ productivity, the search for DNA markers associated with the RJ productivity and a queen selection program to increase RJ production is of considerable scientific and practical interest (Cao *et al*., 2016; Ostrovski-Tomporoski *et al*., 2016).

MATERIAL AND METHODS

We investigated the genetic diversity of the *mrjp3* microsatellite locus in 806 honeybees from 97 bee colonies inhabiting Siberia, Russia (from five to fifteen individuals from each bee colony were examined). Using the mtDNA analysis (variability of the locus COI-COII) and morphometric analysis (parameters of wing), the origin of each colony was determined (Ostroverkhova *et al*., 2015, 2016). Bee colonies have a different origin (*Apis mellifera mellifera*, evolutionary branch M; *Apis mellifera carnica* and *Apis mellifera carpatica*, evolutionary branch C), but similar economically significant indicators, including the royal jelly productivity. Identified hybrid families were excluded from the analysis. Thus, an analysis of the variability of the microsatellite *mrjp3* locus was performed in 575 bees.

DNA isolation and polymerase chain reaction (PCR) was carried out according to standard techniques. To amplify the microsatellite *mrjp3* locus, previously proposed sequences of primers were used (Albert *et al*., 1999). Amplification products were analyzed with ABI Prism 3730 Genetic Analyzer and GeneMapper Software (Applied Biosystems, Inc.) according to the manufacturer's recommendations. Allelic frequencies with standard error were calculated using the POPGENE 1.31 software (Yeh *et al*., 1999).

RESULTS AND DISCUSSION

In our study of the genetic diversity of several honeybee subspecies (evolutionary branches M and C) inhabiting Siberia, Russia, the predominate alleles in different subspecies were determined (Table). In the study of Siberian bee colonies, we have shown that allele "529" is characteristic of *A. m. mellifera*. This allele is registered with a high frequency (76%) in the dark-colored forest bees of the Yenisei population (Krasnoyarsk Territory), which is a unique isolated population that has existed for more than 60 years in forest without the importation of new honeybees. In bees of evolutionary branch C (*A. m. carnica*, *A. m. carpatica*) the allele "529" has been rare. On the contrary, alleles "406" and "518" are characteristic for bees of evolutionary branch C, but are rare in *A. m. mellifera* (evolutionary branch M).

Table. The frequency of registration (with an error) of predominate alleles in honeybees from Siberia, Russia

Allele (bp)	Subspecies of honeybee*			
	Apis mellifera mellifera		*A. m. carpatica*	*A. m. carnica*
	Evolutionary branch M		Evolutionary branch C	
	Tomsk region	Krasnoyarsk Territory	Tomsk region	Kemerovo region
406	0.0234±0.0062	0	0.6136±0.0424	0.1379±0.0453
437	0.0753±0.0108	0.1630±0.0194	0.0455±0.0181	0
518	0.0485±0.0088	0	0.1591±0.0318	0.4138±0.0647
529	0.7625±0.0174	0.7597±0.0225	0.0455±0.0181	0.0517±0.0291
Other alleles	0.0903±0.0056	0.0773±0.0066	0.1363±0.0175	0.3966±0.0572
Number of studied bees	299	181	66	29

* The subspecies were determined by morphometric analysis (wing venation) and mtDNA analysis (locus COI-COII) (for details see references by Ostroverkhova *et al*, 2015, 2016). Hybrid bee colonies are not included in the analysis.

In one of the first studies on search for DNA markers associated with the RJ productivity in Africanized bee colonies (*mrjps* loci were analyzed), an association with the alleles C, D, and E of the *mrjp3* locus was found; bees having genotypes DE, DC, and EC were most productive (Baitala *et al*., 2010; Parpinelli *et al*., 2014). In these studies, despite the process of the accumulation of homozygous genotypes in the *mrjp3* locus during selection of highly RJ productive bee colonies, the high heterozygosity is shown for the *mrjps* loci in total.

Studies of microsatellites for *Apis mellifera ligustica* have shown high genetic diversity, high levels of heterozygosity, and significant genetic differentiation both between the highly RJ productive *A. m. ligustica* lineage from different regions of China and between different populations regardless of their productivity (Chen *et al*., 2005; Ji *et al*., 2009; Yin *et al*., 2011). These data on level of heterozygosity is consistent with values of the average heterozygosity observed for the three *mrjps* loci (*mrjp3*, *mrjp5* and *mrjp8*) in Africanized bees (Baitala *et al*., 2010; Parpinelli *et al*., 2014).

Thus, before using the microsatellite *mrjp3* locus for selection of a highly RJ productive bee colonies, it is necessary to answer the following questions. (1) Do the results of the association of some alleles of the *mrjp3* locus with the RJ productivity of bee colonies reflect universal pattern or represent a particular case characteristic for Africanized bees? (2) Can the microsatellite *mrjp3* locus be considered as a potential DNA marker differentiating subspecies? (3) Does the high level of heterozygosity on both the *mrjps* loci and the entire genome determine the RJ productivity of bee colonies?

To answer these questions, a gene-geographical analysis, a study of the genetic diversity of different bee subspecies and bee colonies with different economically significant indicators, and determining the influence of environmental factors on the genetic diversity of bee subspecies and bee colonies with different productivity are necessary.

These data are important for the development of selection programs for the breeding of bee colonies with genetic characteristics that determine the high RJ productivity.

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